

## *Candida* species and antifungal resistance

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### Abstract

The emerging of *Candida* non-albicans species and the emerging of resistant strains to antifungals is an important threat for population and puts serious problems for medical personnel and for responsible from public health authorities. We investigate structure of *Candida* species from different infections from two hospitals from Bucharest Romania, and we tested them for antifungal resistance. We found *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilopsis*, *C. lusitaniae* species. From *Candida* strains we found about 20% to be resistant to antifungals, some of them being multiple antifungals resistant.

**Key words:** *Candida* infections, resistance to antifungals, *Candida* non albicans species

### Introduction

The yeasts *Candida* are one of the most frequent fungal infections. Most frequent species is *C. albicans*, but there are other species too, like *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* [1]. Other less frequent are *Candida guilliermondi* [2], *Candida stellatoidea* [3], *Candida dubliniensis* [4], *Candida famata* [5], *Candida lusitaniae* [6], *Candida africana* [7], *Candida pseudotropicalis* [8], *Candida kefyr* [9], *Candida rugosa* [10] and *Candida lambica* [11]. The factor of risk of *Candida* infections are: following treatment long time with antibiotics [12], treatments which are producing immunosuppression [13], malignant diseases [14], liver diseases [15], obesity [16], human immunodeficiency virus (HIV) positives [17], patients with trauma [18], long hospital stay [19], diabetes [20], alcoholism [11], malignant blood diseases [21], tuberculosis [22]. Other risk factors are: burns [23], kidney failure [24], mechanical ventilation [25], venous catheters [26], or urinary catheters [27], haemodialysis [28].

There are many reports on the *Candida* species in different types of infections. The vaginal infections reveal that not always the prevalence of *C. albicans* is the most important (32.4%), but other were in great presence, like *C. parapsilopsis* (45%), *C. glabrata* 22.53% [29]. Other study on 350 women between 16 and 45 years old, reveal an emergence of other strains – *C. albicans*-74.4%, *C. glabrata* 9.11%, *C. tropicalis* with 5.6%, *C. krusei*-3.36%, and

*C. parapsilopsis* and *C. guilliermondi* with 2.2% each [30].

There are some targets of the antifungal agents azoles– inhibition of macromolecular synthesis; flucitozine, impair membrane permeability; polyenes, inhibit ergosterol synthesis (azoles derivatives and others), and microtubules from the cells – griseofulvin [31]. The same author, show all the mechanism and targets of the action of antifungal – the nucleic acid synthesis, the ergosterol synthesis, the protein synthesis and so on. One of the most common infections is the oropharyngeal candidiasis and this can be a real problem when we deal with fluconazole resistant strains. Fluconazole is known to inhibit the ergosterol synthesis and the resistance occurs in the case of changes in ergosterol by mutations in the drug target enzymes, the sterol 14 a demethylase, or by changes in expression of genes coding for membrane transport proteins [32]. Same alteration of the sterol biosynthesis, by mutation of the drugs target enzyme, the sterol 14 demethylase (14 DM) which results in a lower affinity for fluconazole of the sterols [32, 33].

Another source is the gene MDR1, which express the major facilitator family, and it is over expressed in isolates resistant to fluconazole. The author declares that the MDR1 gene over expression is a source of multidrug resistance [33]. There were strains identified as multidrug resistant in hospitals

[34]. The multidrug resistance can be practically the results of multiple mutations [35].

Not only resistant strains to azoles antifungal, but strains resistant to echinocandins were also isolated [36]. Some authors show that the echinocardine resistance is increasing [37], and this is an important threat for the near future, in specially when the fungi are resistant to different classes of antifungals [38]. Some specialists [40] reviews the problems related with *Candida* infections, one of the most important being the resistance of hospital strains to antifungal agents. The authors showed morphological and clinical aspects of *Candida* strains and reviewed the mechanism of resistance. One of the biggest risks in hospital for immunocompromised patients is candidemia – invasive *Candida* infections-from commensals, becoming pathogenic.

### Methods

Two lots of patients were investigated, one from Elias Hospital (156 cases) and one from CFR2 Hospital (Hospital of the Romanian Railway National State Company) with a number of 134 cases both from Bucharest. The samples were collected by specialized personnel and analysed in hospital microbiology laboratory. Samples (oropharyngeal, urogenital, vulvovaginal) were obtained from patients and analysed in laboratory by classical methods - cultivation on Chromogenic culture media for *Candida* (Chromatic Candida Agar, MLT, Arad, Romania), kit tests Candifast™ (Elitech SRL, Bucharest Romania) a test which is used both for identification of *Candida* species and determination of their susceptibility to six main antifungals (Amphotericin B, Nystatin, Fluconazole, Fluocytosine, Econazole, Ketoconazole Myconazole), currently used in practice of treatment of fungal infections. Data about the patients' anamnesis and others were obtained from medical records of the hospitals.

### Results and comments

The species isolated from the infections diagnosed in Elias Hospital were *C. albicans* (76.9%), 21 cases infections with *C. glabrata* (13.5%), 13 cu *C. krusei* (8.4%) and 2 with *C. parapsilopsis* (1.3%) (fig. 1). From the CFR2 Hospital resulted a number of 134 cases of *Candida* infections from which 121 with *C. albicans* (90.2%), 2 with *C. glabrata* (1.5%), 9 with *C. krusei* (6.7%), 2 with *C. parapsilopsis* (1.5%) and 1 case with *C. lusitaniae* (0.7%) (fig 2).

*C. albicans* was the predominant specie in these infections with 70% in Elias Hospital and respectively in CFR2 Hospital 90% from the isolates.

At the Elias Hospital, *C. albicans*, a number of 31 were resistant to antifungals (19.8%), majority- 18 isolates rezistants to an antifungal (32.2%), 7 strain (22.5%) to two antifungals, 4 strains- at three antifungals (12, 9 %) and 2 strains to four antifungals (6.5%) described in Figure 3.

In CFR 2 Hospital were identified 33 resistant strains (24.6%) 12 strains resistant to 1 antifungal (36.4%), 13 strains to 2 antifungals (39.3%), 6 to 3 antifungals (18.18%) and 2 strains to 4 antifungals ( 6.06%) (fig.4).

There were many cases of resistance to one or more antifungals. Most frequent were the ones resistant to Miconazol, and then to Amphotericin B, Fluconazol, Econazol and the less frequent the strains resistant to Ketoconazol, Flucitazine and Nystatin. Some other resistant strains were from species *C. krusei* and *C. glabrata* but no resistant strains in the case of *C. parapsilopsis* and *C. lusitaniae* could be detected (fig.5).

The patients suffered of diseases or states which probably weakened the immunity. At Elias hospital the patients' situations were: a total of 156 patients, 37 men and 119 women (fig.6). There were 70 cases of pregnancy, arteriopathy 1 case, anaemia and haemoptysis 4 cases, 36 cases carcinoma, 12 cases stroke, sepsis 6 cases, diabetes, pneumonia 3 cases, gastric adenocarcinoma 2 cases, cirrhosis 1, asthma 1, Cushing syndrome 1, epilepsy 1 case. In CFR 2 Hospital the situation was as following: 134 patients, 7 men and the rest, women (127) (fig.8). The states or the diseases of the patients were inflammatory pelvic inflammations (101 cases), other inflammations of vulva and vagina (2 cases), inflammations of cervix (2 cases), pharyngitis and laryngitis and acute tonsillitis (6 cases), other diseases of respiratory superior tract (5 cases), acute rhinopharyngitis (3 cases), anaemia, iron deficiency (1 case) and non-insulin-dependent diabetes (1 case) (fig.9). The age structure was at Elias Hospital (age groups): 2 patients 10-20 years old (y.o.), 21-30 y.o. (46 patients), 31-40 y.o. (36 patients), 41-50 y.o. (2 patients), 51-60 y.o. (19 patients), 61-70 y.o. (22 patients), 71-80 y.o. (15 patients), over 80 y.o. (15 patients). At CFR2

Hospital the age structure was: under 10 y.o. (9 patients), 11-20 y.o. (15 patients), 21-30 y.o. (37 patients), 31-40 y.o. (34 patients), 41-50 y.o. (23 patients), 51-60 y.o. (8 patients), 61-70 y.o. (6 patients), 71-80 y.o. (2 patients), over 80 y.o. (1 patient) (fig.7).

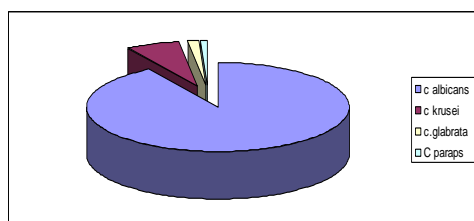


Fig. 1. Structure of *Candida* species isolated from infections from Elias Hospital 1- *Candida albicans*; 2- *Candida krusei*; 3- *Candida glabrata*; 4- *Candida parapsilopsis*

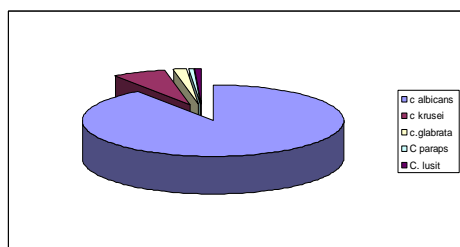


Fig. 2. Structure of *Candida* species isolated from infections from CFR2 Hospital: 1- *Candida albicans*; 2- *Candida krusei*; 3- *Candida glabrata*; 4- *Candida parapsilopsis*; 5- *Candida lusitanae*.

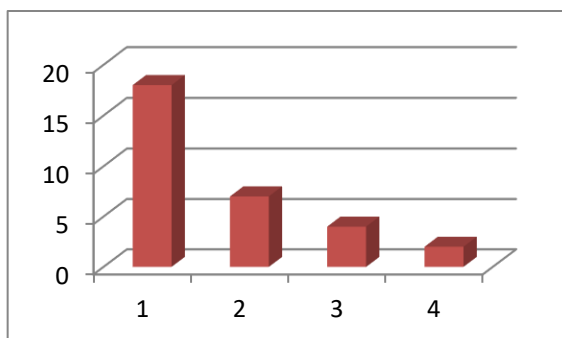


Fig 3. Number of strains of *Candida albicans* isolated from patients from ELIAS Hospital resistant to 1, 2, 3 and 4 antifungals

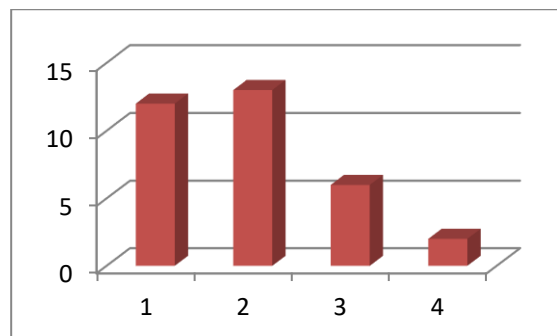


Fig 4. Number of strains of *C. albicans* isolated from patients from Hospital CFR 2 resistant to 1, 2, 3, and 4 antifungals

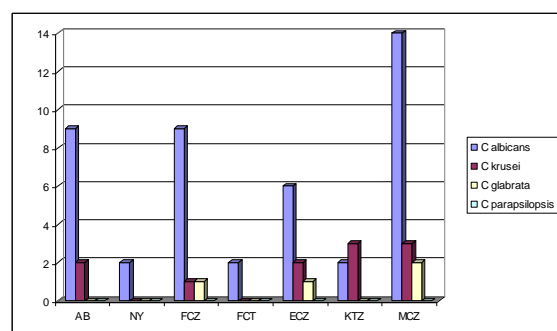


Fig.5. Strains *C. albicans* and non-*albicans* resistant to antifungals AB-amphotericin B; NY-nystatin; FCZ-fluconazole; FCT-flucitonazole; ECZ-econazole; KTZ-ketoconazole; MCZ-miconazole.

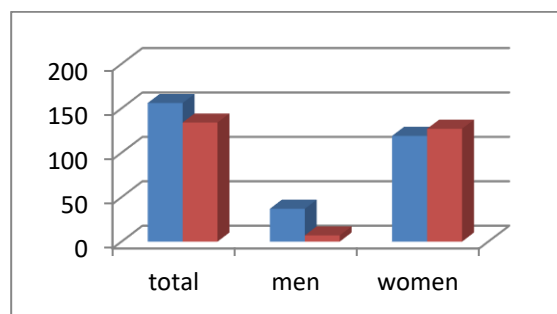


Fig. 6. Structure of the patients by sex in the investigated hospitals (blue – Elias Hospital; red - CFR2 Hospital)

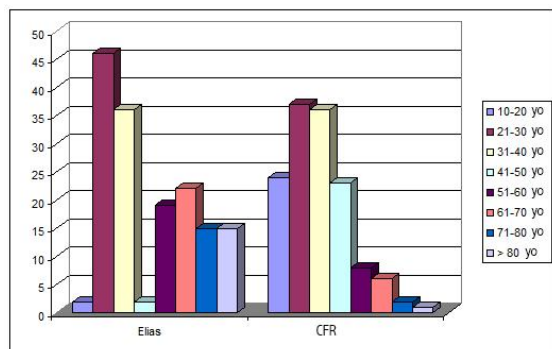


Fig. 7. Age groups structure in both hospitals of the investigated patients

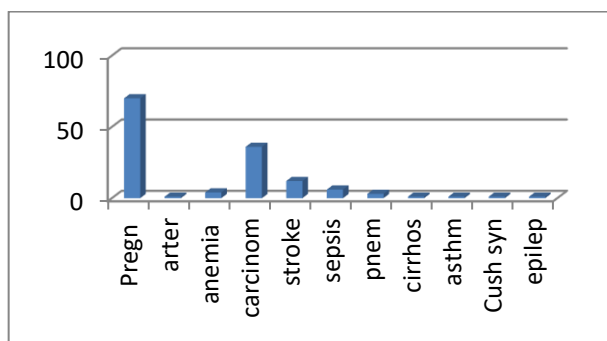


Fig. 8. Case of different diseases and states of the investigated patients from ELIAS Hospital Pregn.-pregnancy; arter.-arteriopathy; anemia; carcinoma.-carcinoma; stroke; sepsis; pnem-pneumonia; cirrhos-cirrhosis; asthm-asthma; Cush. Syn.-Cushing syndrome; epilep.-epilepsia

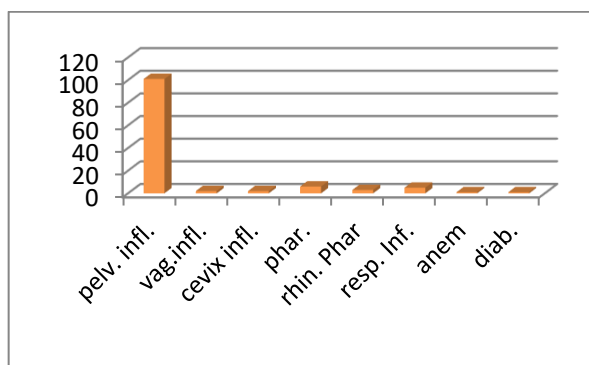


Fig. 9. Cases of different diseases and states of investigated patients from CFR2 Hospital (pelv infl. – pelvic inflammation; vag. Infl – vagina inflammation ; cervix infl. –cervix inflammation; phar-pharyngitis; rhin. Phar – rhino- pharyngitis; resp inf. –respiratory infections; anem-anemia; diab.- diabetes).

## Conclusions

The structure of *Candida* species and strains are very complex, besides *C. albicans* there are other strains contributing to the fungal infections, but less frequent (*C. krusei*, *C. glabrata*, *C. parapsilopsis*, *C. lusitaniae*).

From the total *C. albicans* strains, a percentage of 19.8-24.6% were resistant to antifungals. About 6.5% were resistant to four antifungals. This is a real threat for patients, and could be a problem for public health authorities.

The patients were women in state of pregnancy or persons with diseases or conditions which predisposes to a weakened immunity.

Further investigations should be done to establish new antifungals with high efficiency and better prevention measures.

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